CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

National Cancer Institute Clinical Proteomic Technologies Initiative for Cancer

RFA-CA-07-005 and RFA-CA-07-012

Gregory J. Downing, D.O., Ph.D. and Adam M. Clark, Ph.D.

February 27, 2006

http://proteomics.cancer.gov

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Agenda

10:00 a.m. - 10:05 a.m.	**Welcome and Introductions** *Gregory J. Downing, D.O., Ph.D., OTIR, NCI, NIH*
10:05 a.m. - 10:20 a.m.	**Proteomics and Advanced Technologies in Cancer Research** *Anna D. Barker, Ph.D., Deputy Director, NCI, NIH*
10:20 a.m. - 12 noon	**Description of the Clinical Proteomic Technology Assessment for Cancer RFA** *Gregory J. Downing, D.O., Ph.D., OTIR, NCI, NIH* *Henry Rodriguez, Ph.D., OTIR, NCI, NIH* *Adam Michael Clark, Ph.D., OTIR, NCI, NIH*
12 noon - 1:00 p.m.	**Lunch** *(onsite cafeteria)*

Agenda (continued)

1:00 p.m. - 1:15 p.m.	**Intellectual Property** *Thomas Stackhouse, Ph.D., TTB, NCI, NIH*
1:15 p.m. - 1:30 p.m.	**Application and Review** *Sherwood Githens, Ph.D.,* *Division of Extramural Activities, NCI, NIH*
1:30 p.m. - 1:50 p.m.	**Sample Collection and Biospecimens** *Jim Vaught, Ph.D., OBBR, NCI, NIH*
1:50 p.m. - 2:10 p.m.	**caBIG** *George Komatsoulis, Ph.D., caBIG, NCI, NIH*
2:10 p.m. - 3:00 p.m.	**Questions – Public Forum**

Webcast Viewers

- **Email questions during the presentation to nciproteomics@yahoo.com**
- **Archived viewing will be available through the website http://proteomics.cancer.gov**
- **Sign up for initiative updates at the website at http://proteomics.cancer.gov/email_signup.asp**

proteomics.cancer.gov

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Opportunities and Issues in Proteomics

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Challenge of Cancer

- **Problem:**
  - Cancer metastasizes before it can be detected
  - Tumors are difficult to control
- **Potential Solution:**
  - Protein-based detection and monitoring of cancer processes
- **Challenges**
  - Detection of low abundance proteins
  - High sensitivity and specificity
  - Label-free detection
  - High-throughput platform analysis
  - Clinical application

Community Input and Plan Development

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April 2002

April 2003

June 2004

Sept 2004
Nov 2004

Jan 2005

Feb 2005

- **Proteomics Planning Workshop (NCI/NHGRI/NIGMS)**
  Bethesda, Maryland
- **Proteomic Technologies for Early Cancer Detection**
  Chantilly, Virginia
- **Initial draft proposal for a Clinical Proteomics/ Biomarker Discovery Initiative**
- **Clinical Proteomics and Biomarker Discovery in Cancer Research**
  East Coast – Bethesda, Maryland
  West Coast – Menlo Park, California
- **Clinical Proteomics Technologies Team Initiative proposal**
- **Proteomic Technologies Informatics Workshop**
  Seattle, Washington

Community Input and Consensus

- **On the basis of discussions with a wide range of clinicians, cancer researchers, and technologists, the NCI recognizes that there are immense opportunities for using proteomics technologies to solve mission-critical problems in cancer research.**

- **Premises:**
  - Biomarkers exist in readily-accessible body fluids
  - Panels of such markers will be required to achieve high specificity and sensitivity
  - Current technology is capable of discovering these panels
  - Current application of this technology can be improved

Incorporating BSA Input

- **Increased collaboration for standards development, reagent and resource support, and protein production**
- **Developed programmatic requirements to ensure integration of technology and data analysis enhancement**
- **Added statistical and epidemiology expertise to Program Coordinating Committee**
- **Included common mouse model of human cancer to inform technology improvements and protocol development**

Since no current technology interrogates more than approximately 1% of the proteome at a time, a systematic approach to biomarker discovery requires teams of investigators who share and aggregate data.

Clinical Proteomic Technologies Initiative for Cancer (CPTI)

- **NCI Clinical Proteomic Technologies Initiative for Cancer represents a highly-organized approach to apply proteomic technologies and data resources to support the discovery of clinical biomarkers for the early detection of cancer and to monitor therapeutic outcomes.**

- **CPTI teams will support the development of standards and resources for a clinical proteomics platform for cancer research by:**
  - Harnessing team efforts to establish standards;
  - Ensuring rigorous quality control measures; and
  - Developing an informatics platform in collaboration with caBIG that is capable of aggregating and comparing data across laboratories.

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CPTI: Strategy and Programs

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CPTI Program Implementation

- **Areas of Technology Development and Assessment**
  - Sample Preparation and Labeling
  - Sample Fractionation
  - Mass Spectrometry
  - Protein Capture and Microarray
  - Data Analysis and Bioinformatics
- **Advanced Proteomic Platforms and Computational Sciences**
  - RFA-CA-07-005; R01, R21/R33 Funding Mechanisms
  - Designed to support highly innovative research in the quantitative analysis of peptides/proteins of interest in clinical cancer studies
- **Clinical Proteomic Technology Assessment for Cancer**
  - RFA-CA-07-012; U24 Funding Mechanisms
  - Designed to improve proteomic analysis platforms to reliably identify, quantify, and compare peptides/proteins in complex biological mixtures

The Human Plasma Proteome: History, Character, and Diagnostic Prospects

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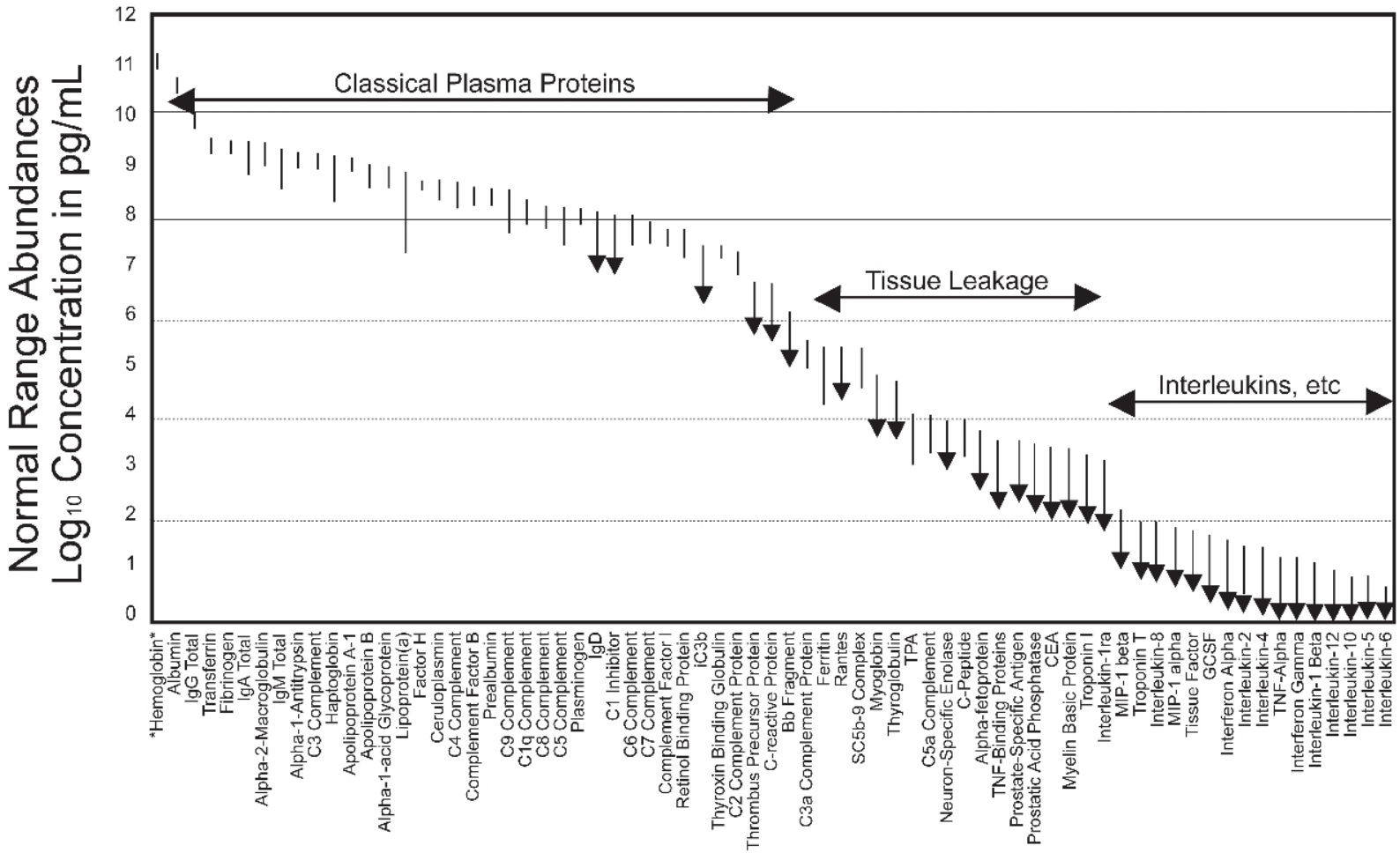
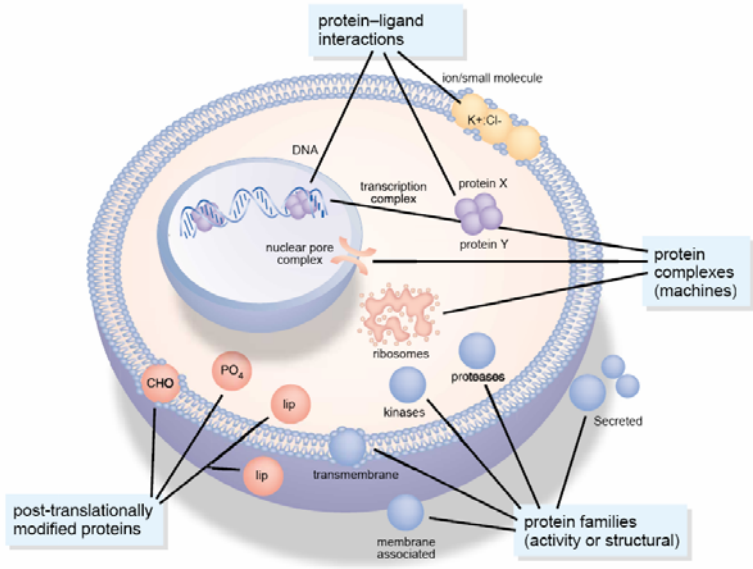
Figure: Reference intervals for 70 protein analytes in plasma.



Source: Anderson, N. L., and Anderson, N. (2002) The Human Plasma Proteome: History, Character, and Diagnostic Prospects. *Molecular & Cellular Proteomics*, **1,** 845-867.

Proteomics Today

- **No** single technology platform that can satisfy all of the desired proteomic measurements
- **No** mature, “true” proteomic technology
- **No** performance criteria
  - Poor confidence in protein measurement results
  - Difficulty in assessing agreement of different experiments
  - Conflicting reports in the literature
  - Lost opportunities



Scott D. Patterson & Ruedi H. Aebersold, Proteomics: the first decade and beyond, *Nature Genetics* 33, 311-323 (2003)

If proteomics technologies are to successfully make their way into clinical diagnostics, universally accepted metrics will be needed at many steps along the way to help clarify experimental results and protocols and make them comparable.

Opportunities in Proteomic Cancer Research

NCI encourages partnerships and collaborations between biologists, clinicians, statisticians, and computer scientists

- Critical Components in Clinical Cancer Proteomics
  - Cancer biology
  - Clinical research
  - Statistical design, analysis, and metrology
  - Technologies
  - Bioinformatics and computational sciences
- Areas of Interest
  - Peptide/Protein identification and quantification
  - Detection of post-translational modifications, splice variants, and mutations
  - Improved statistical measures of confidence
  - Data normalization
  - Standard reagents, resources, and protocols

Challenges Exist to the Clinical Measurements of Proteins

- **Pervasive problems with research design, data analysis, reproducibility, and comparability of research results**
- **Lack of common reagents and highly qualified public data sets**
- **Ineffective and inefficient transfer of platform technologies to clinical application**
- **Inability to manage and interpret large quantities of pre-processed data**
- **Private sector unable and unlikely to address the challenges**

Challenges to Technology Assessment and Data Analysis

- **Data validation and reproducibility**
  - Instrument calibration and comparison
  - Peptide/Protein identification
  - Quantification
  - Data normalization
  - Annotation and ontologies
- **Statistical considerations and planning**
  - Chance versus reproducibility
  - Bias
  - “One-hit wonders”
  - Non-disease and/or co-morbid proteomic evaluation references

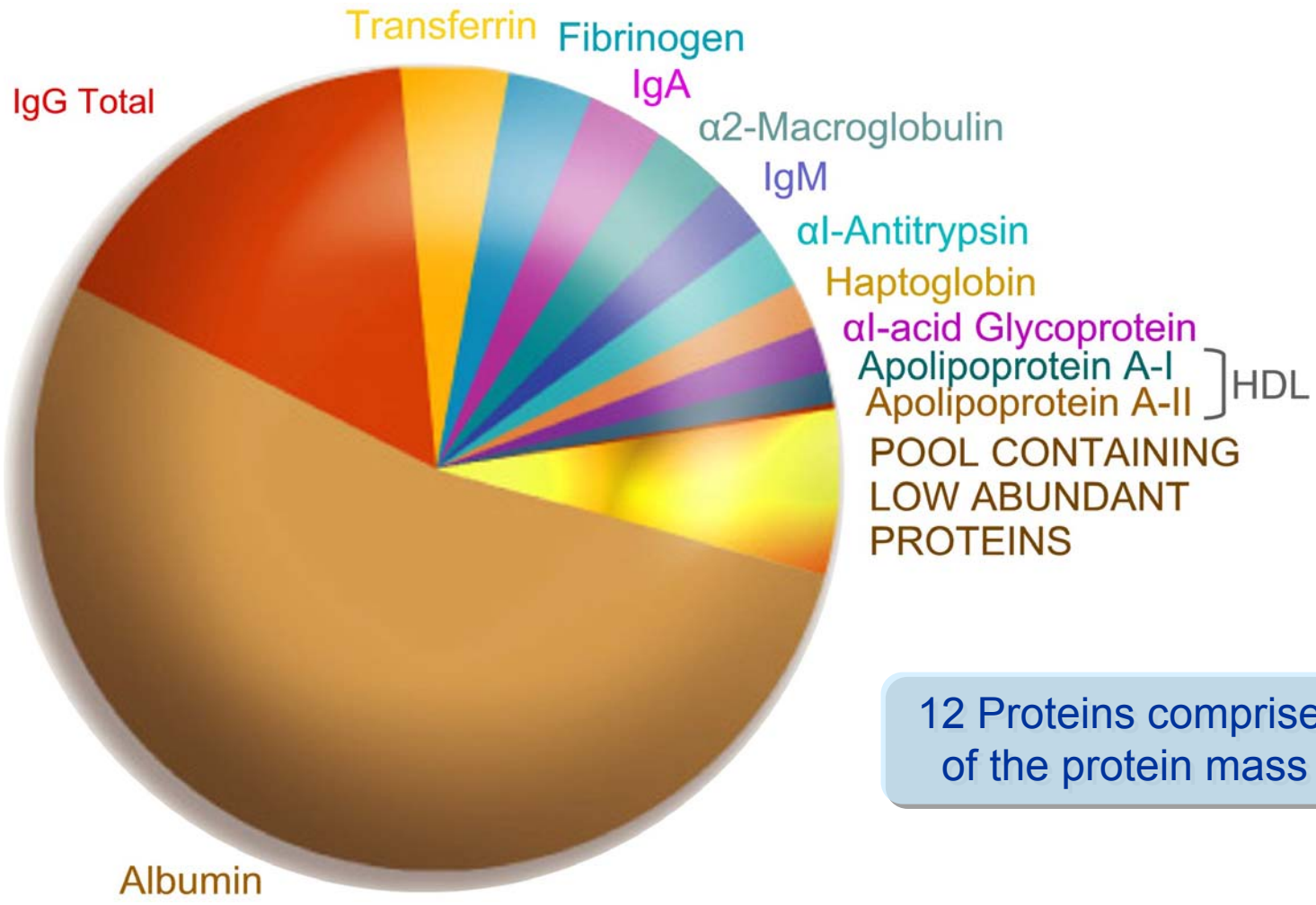
Sources of Variability of Existing Proteomic Technologies

- **Specimen handling and processing**
- **Platform evaluation**
  - Technical (resolution, accuracy, dynamic range, sensitivity, reproducibility)
  - Cross verification among platforms
- **Data acquisition/Bioinformatics**
- **Data analysis**
- **Publication uniformity**

Goal: Assurance that protein measurement results are due to changes in the sample and not changes or variability due to:

- Instrument
- Assay performance
- Reagents
- Operator
- Site

Clinical Proteomic Technologies Initiative Strategy



12 Proteins comprise up to 96% of the protein mass in plasma

Clinical Proteomic Technologies Initiative Approval and Development

Jan 2005	• NCI Executive Committee Proteomics Concept Approval
June 2005	• NCI Board of Scientific Advisors Review and Approval of the Clinical Proteomic Technology Initiative for Cancer
Nov 2005	• Publication of RFA-CA-07-005 Advanced Proteomic Platforms and Computational Sciences
Dec 2005	• Proteomic Technologies Reagents Resource Workshop
Feb 2006	• Publication of RFA-CA-07-012 Clinical Proteomic Technology Assessment for Cancer
Apr 11, 2006	• Expiration Date for RFA-CA-07-005
Apr 21, 2006	• Expiration Date for RFA-07-012
Summer ’06	• Review and Awards
Fall ’06	• Standards and Reagents Incorporation

Overcoming Technical Barriers

- **Build a multidisciplinary team framework**
  - To permit large-scale, real-time exchange and application of existing and newly development protein measurement technologies, biological resources, and data dissemination
- **Refine and standardize technologies, and statistical and analytical methods**
  - To ensure reliable and reproducible separation, capture, identification, quantification, and validation of protein measurements
- **Develop and evaluate new technical approaches**
  - To separate and recognize proteins of clinical significance

Proteomics Experimental Design: Standards, Metrics, and Variability

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- **Statistical and epidemiological consultants to advise on experimental design, processes, and reporting**
- **Program management and coordination**
  - Assessment of objectives versus study design and measurements
  - Certified standards and samples for calibration, bias, and uncertainty measures
    - Matrix
    - Peptides, proteins
  - Experimental design and comparison
  - Data standardization for sharing information (caBIG)
- **Control of systematic sources of variation**
  - Sample characteristics (e.g., age, gender, diet)
  - Sample handling and storage conditions
  - Instrument conditions and characteristics
- **Control of random variation**
  - Adequate sample sizes to control between sample variation
  - Replication of measurements on a given sample
- **Calibration of platform performance for laboratory comparison studies**

CPTI: Expected Results

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- **Creation of public resources**
  - Reference sets
  - Reagents
  - Protocols
  - Algorithms and databases
- **Accelerated protein-related discovery research and applications**
- **Enhanced knowledge base to support discovery and translational research**
- **Well-characterized and documented candidate-based approaches for peptide/protein identification**
- **Technology Development and Assessment:**
  - Sample Preparation and Labeling Technologies
  - Sample Fractionation Technologies
  - Mass Spectrometry and Microarray/Protein Capture Technologies
  - Data Analysis
  - Microsimulation
  - Validation

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Funding Mechanisms and Process

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Components of the Initiative

proteomics.cancer.gov

Funding Mechanisms ($100+ million)

- **I. Clinical Proteomic Reagents Resource**
- **II. Advanced Proteomic Platforms and Computational Sciences**
  - Next generation technologies
  - R01, R21/R33 ARD: 04/11/06
- **III. Clinical Proteomics Technology Assessment for Cancer (CPTAC)**
  - Evaluate existing technologies
  - U24 ARD: 04/21/06

Revised Funding Plan
FY 2006 – 2010 ($ in millions)

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Program Activity	Mech	Est. # Awards	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Clinical Proteomic Technology Assessment	**U24**	3-5	**8.5**	**8.5**	**8.5**	**5.0**	**5.0**	**35.5**
Advanced Proteomic Platforms and Computational Sciences	**R01, R21/R33**	10	**10.0**	**12.0**	**15.0**	**14.0**	**5.0**	**56.0**
Clinical Proteomic Reagents Resource	**RFP contracts**		**2.5**	**2.5**	**2.5**	**2.5**	**2.5**	**12.5**
Estimated Overall Funding TOTAL			21	23	26	21.5	12.5	**104**

I. Clinical Proteomic Reagents Resource

- **Role:**
  - To serve the investigator community as a central public resource for well characterized proteomic reagents and resources
- **Key Features:**
  - Develop standard and characterized reagents (antibodies, proteins, and peptides)
  - Develop appropriate quality assurance/quality control procedures
  - Provide an interactive resource “catalog” through caBIG
  - Expedite acquisition and distribution of reagents
  - Provide data on reagent performance
- **Components:**
  - Well-characterized peptides (SDS-PAGE, mass spectrometry, circular dichroism, and light scattering)
  - Standard reference materials
  - Well-characterized antibodies and affinity capture reagents

II. Advanced Proteomic Platforms and Computational Sciences

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RFA-CA-07-005; R01 and R21/R33 funding mechanisms

- **Role:**
  - To support development of innovative tools and technologies for protein/peptide measurement; support algorithm development and computational methods
- **Key Features:**
  - Technology development in analytical methods and computational sciences
    - Improve protein and peptide measurement, identification, and characterization
    - Experimental validation
    - Data analysis
    - Interrogation of large pre-processed data sets
  - Integrated tools and data resources
  - Clinical utility

http://grants1.nih.gov/grants/guide/rfa-files/RFA-CA-07-005.html

II. Advanced Proteomic Platforms and Computational Sciences

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- **(R21/R33) Current technology platforms have various performance endpoints that can be improved, including dynamic range, mass accuracy, sample throughput, peptide/protein identification, quantification, reproducibility, and cost.**
  - Sample Preparation and Labeling Technologies
  - Sample Fractionation Technologies
  - Mass Spectrometry
  - Protein Capture and Microarray Technologies
  - Data Analysis
- **(R01) Computational, statistical, and mathematical approaches for the analysis, processing, and facile exchange of large proteomic data sets**

The RFA is not intended to be proscriptive, but intends to identify innovative research programs able to improve the capabilities of current proteomic technologies and data analysis programs by an order of magnitude.

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Clinical Proteomic Technology Assessment for Cancer (CPTAC)

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III. Clinical Proteomic Technology Assessment for Cancer (CPTAC)

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RFA-CA-07-12; U24 Cooperative agreement funding mechanism

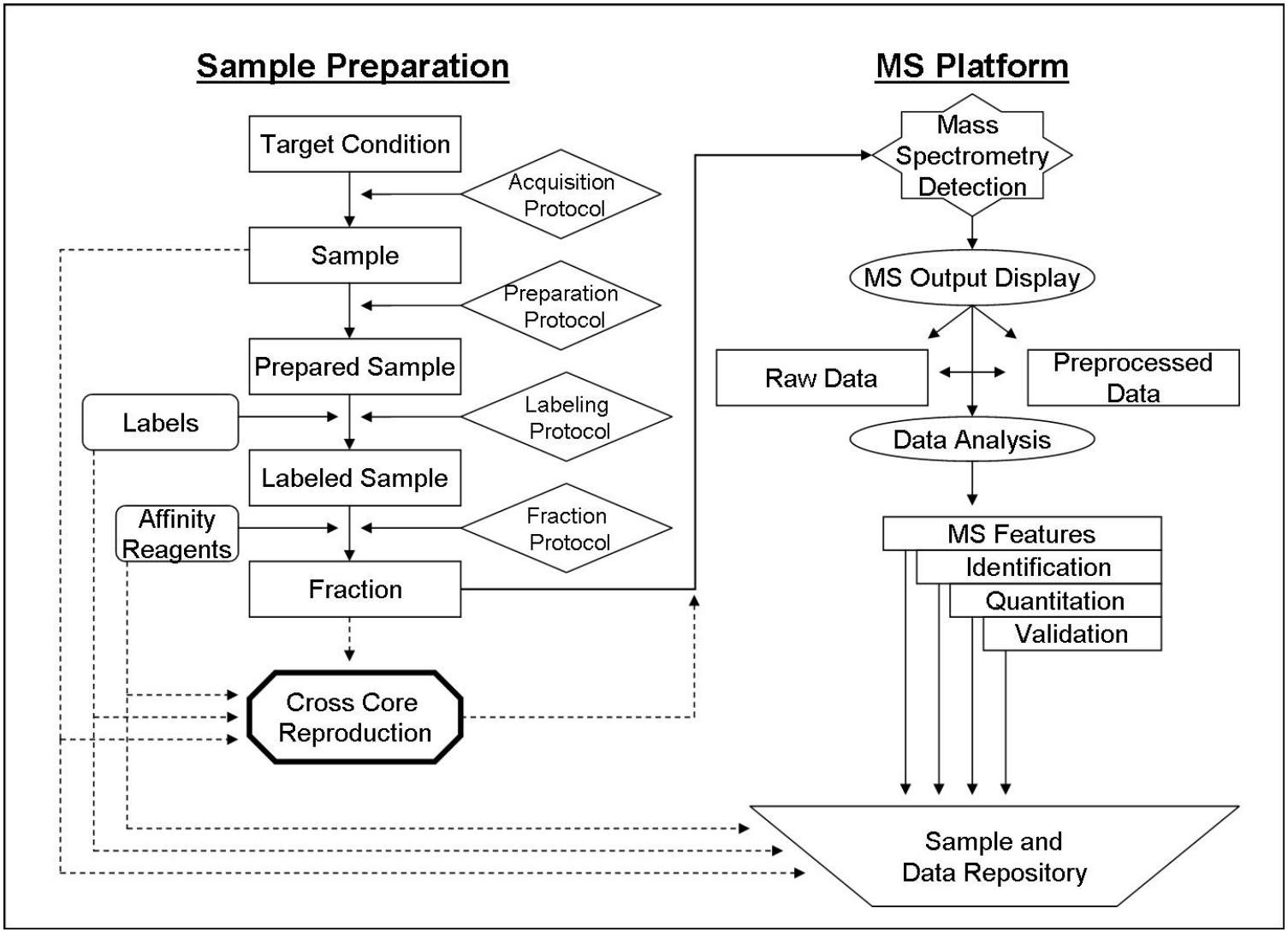
- **Role:**
  - To establish a multidisciplinary network that will conduct rigorous proteomic technology assessment; develop standard protocols and clinical reference sets; and evaluate methods to ensure data reproducibility
- **Key Features:**
  - Assemble expertise needed to evaluate technology platforms with intended applications in clinical discovery research
  - Focus on experimental design, methods, standards development, data analysis, and interlaboratory comparisons
  - Establish highly annotated standards and clinical reference sets
  - Protocol and methods development

http://grants1.nih.gov/grants/guide/rfa-files/RFA-CA-07-012.html

Goals of the CPTAC Program

- **Objective 1:** Evaluate performance of proteomic technology platforms and standardize approaches to developing applications using these platforms;
- **Objective 2:** Evaluate proteomic platforms for their ability to analyze cancer-relevant proteomic changes in human clinical specimens;
- **Objective 3:** Establish systematic ways to standardize proteomic protocols and data analysis among multiple laboratories;
- **Objective 4:** Develop and implement uniform algorithms for sharing bioinformatics and proteomic data and analytical/data mining tools across the scientific community;
- **Objective 5:** Develop well-defined and comprehensively characterized sets of standard/reference materials and reagents to serve as resources for the research community.

Clinical Proteomics: Technology Development and Standardization



Technologies for the CPTAC Program

- **Mass Spectrometry**
  - At least two variants of mass spectrometry platforms (examples of technologies and variants include: MALDI, ESI, TOF, FTICR, tandem MS/MS, and LC-MS
  - Of those, at least one platform must have two instruments
  - 2DGE and SELDI technologies will not be included in this program due to limitations in throughput, reproducibility, and peptide/protein identification
- **Validation and Developing Proteomics Technologies: Affinity Capture and Microarray**
  - Validation studies based on mass spectrometry data
  - Innovative applications
  - Coordinate platforms with the Reagents and Resources Core
- **Partnerships with industrial technology experts encouraged**

Experimental Design

- **Performance Evaluation**
  - Quantification: dynamic range of peptide/protein measurements
  - Sample throughput
  - Specificity
  - Accuracy of peptide/protein identification
- **Reproducibility**
  - Same site
  - Cross site
  - Cross platform
  - Cross technology
- **Identification of cancer-related proteins in clinical samples**
  - 200 individual clinical samples of body fluids with matching tumors
  - Sufficient amount of samples to be shared across the CPTAC teams
  - Candidate-based approaches: signaling pathways, expression data, biological/physiological processes
  - Plans must include provisions for bioinformatics tools/algorithms

Standards, Reagents, and Resources

- **Standardized reference materials and measurement assessment material to be developed in coordination with the program**
- **Standard peptide mixtures and standard biological fluids**
- **Peptides and antibodies will be developed as a component of the Clinical Proteomic Technologies Initiative and applied to the CPTAC program accordingly**
- **It is intended that measurement assessment materials and standard reference materials will be developed through the program along with well-documented measurements and sources of variability**
- **Standardized protocols from biological sample handling and preparation through prepared sample analysis will be developed as key components of the initiative**

Reference Materials for CPTAC Inter-comparison Study

- **Protein Mixtures:**
  - For qualitative (Sept/Oct ’06) and semi-quantitative (Dec ’06) studies
  - Composed of ≈ 20 human proteins
    - Classic serum proteins (i.e., albumin, fibrinogen, CRP , etc.)
    - Known cancer biomarkers (i.e., CEA, PSA-ACT, AFP, etc.)
    - Cancer-related proteins (i.e., TNF-α, VEGF, Calcitonin, etc.)
    - Concentrations ranging from g/L to ng/L
    - Characterized by MS/MS and quantified by amino acid analysis
- **Human Pooled Plasma:**
  - For qualitative (April ’07) and quantitative (Nov ’07) studies
  - Unspiked and spiked with cancer biomarkers and cancer-related proteins of known and verified concentrations
  - Concentration measurement of intrinsic plasma proteins made ongoing

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Integration with Existing NCI Resources

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Biospecimens and Proteomics

- **CPTAC Teams will coordinate practices with the Office of Biorepositories and Biospecimen Research (OBBR) at the NCI**
- **Biospecimen practices are to some degree:**
  - Specimen type-specific
  - Analysis specific
  - Intended use-specific
- **Biospecimen value is related to:**
  - Quality of the specimen
  - Quality of the associated clinical data
  - Ethical, legal, and regulatory limitations on acquisition and use

Biospecimens and the CPTAC

- **Participating teams will work in coordination with the OBBR (http://biospecimens.cancer.gov) to develop and evaluate sample acquisition, storage, and preparation procedures.**
- **Applicants will propose between 1 and 3 cancer sites.**
- **Each team will be required to have at least 200 individual clinical samples of body fluids (with sufficient quantity to be shared) and matching tumor samples.**
- **One common mouse model to be applied across all participating teams. The mouse model will be provided by the NCI.**
- **Applicants are encouraged to include mouse models.**
- **Candidate-based approaches can be evaluated through the mouse model samples to facilitate preclinical testing approaches.**

Bioinformatics and Data Management

- **National Cancer Institute Center for Bioinformatics cancer Biomedical Informatics Grid (caBIG)**
  - Virtual cancer research infrastructure
  - Standardized annotation and format
  - Computational Proteomics Analysis System (CPAS)
- **CPTI data: Open-source, publicly available data for investigators**

CPAS (Computational Portal and Analysis System)

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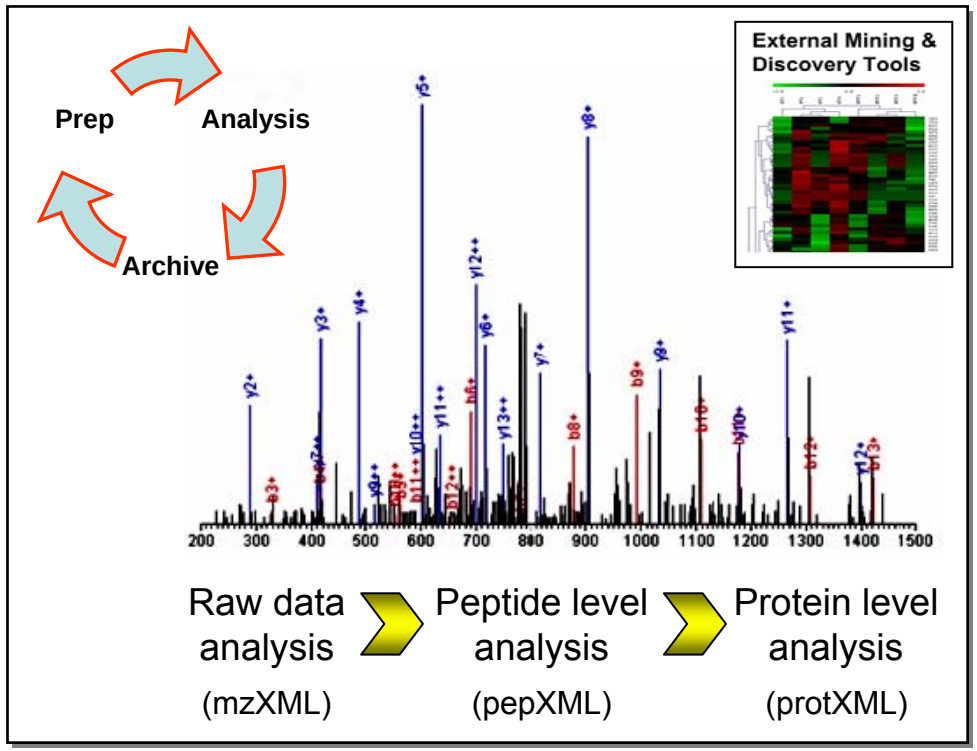
A open source, web-based proteomics data management software suite – combining lab management and informatics modules for high-throughput MS/MS experiments and clinical trials.

- Stores and processes experiment data
- Includes bioinformatics and collaboration modules
- Open-source Java application
- Security controls access to data



Goal:
To help scientists store, analyze, and share proteomics data.

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https://cpas.fhcrc.org

Data Analysis and Informatics

- **Data analysis application will include peptide/protein identification, normalization, quantification, cross platform analysis, and analysis of multiple large data sets.**
- **Algorithms will be developed to be open code and data analysis platforms will be shared within and among the teams.**
- **Data will be stored as preprocessed data and made available to the scientific community through the NCI as the program matures.**
- **Teams will work with caBIG to develop and implement the data at a minimum of “Silver Level” compliance with caBIG.**

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Summary

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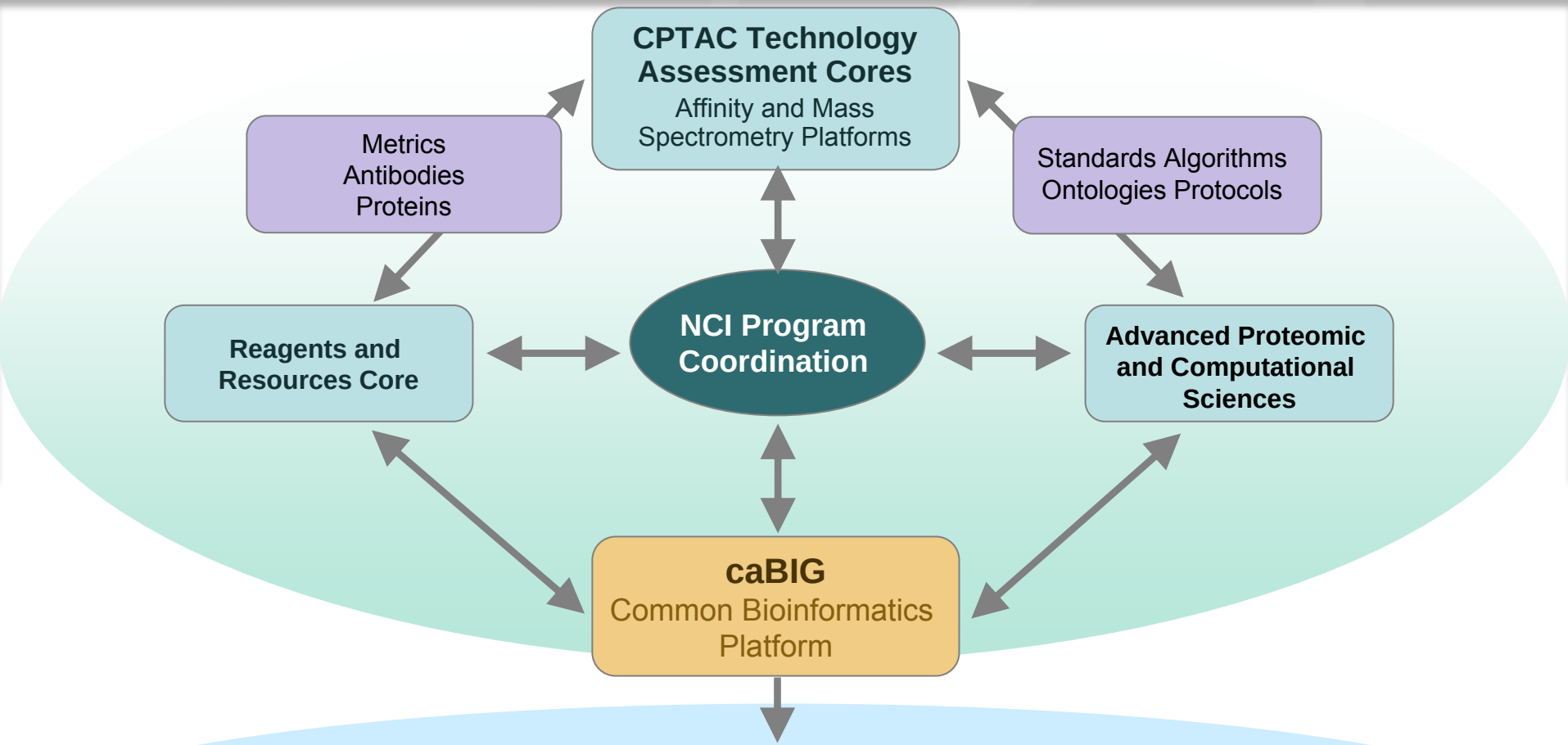
Clinical Proteomic Technologies Initiative Strategy



Community Resources:

- Integrated Searchable Proteomic Database
- Highly Qualified Biospecimens
- Standardized Reagents
- Optimized Technology Platforms
- Proteomic Standards
- New Technologies

Program Coordination

- **Program Coordinating Committee (PCC) will:**
  - Be established as the centralized body of the CPTAC
  - Include all the PIs of the teams as well as members of the NCI to determine appropriate actions
  - Work to align strategies and protocols across the CPTAC and ensure appropriate inter-laboratory studies are properly designed and conducted
  - Prioritize activities, monitor developments, and evaluate research data
- **Voting members of the PCC will consist of the PI (or other designated member) and the NCI Project Coordinator**
- **Additional non-voting members may be added to the PCC to ensure inclusion of appropriate scientific expertise**

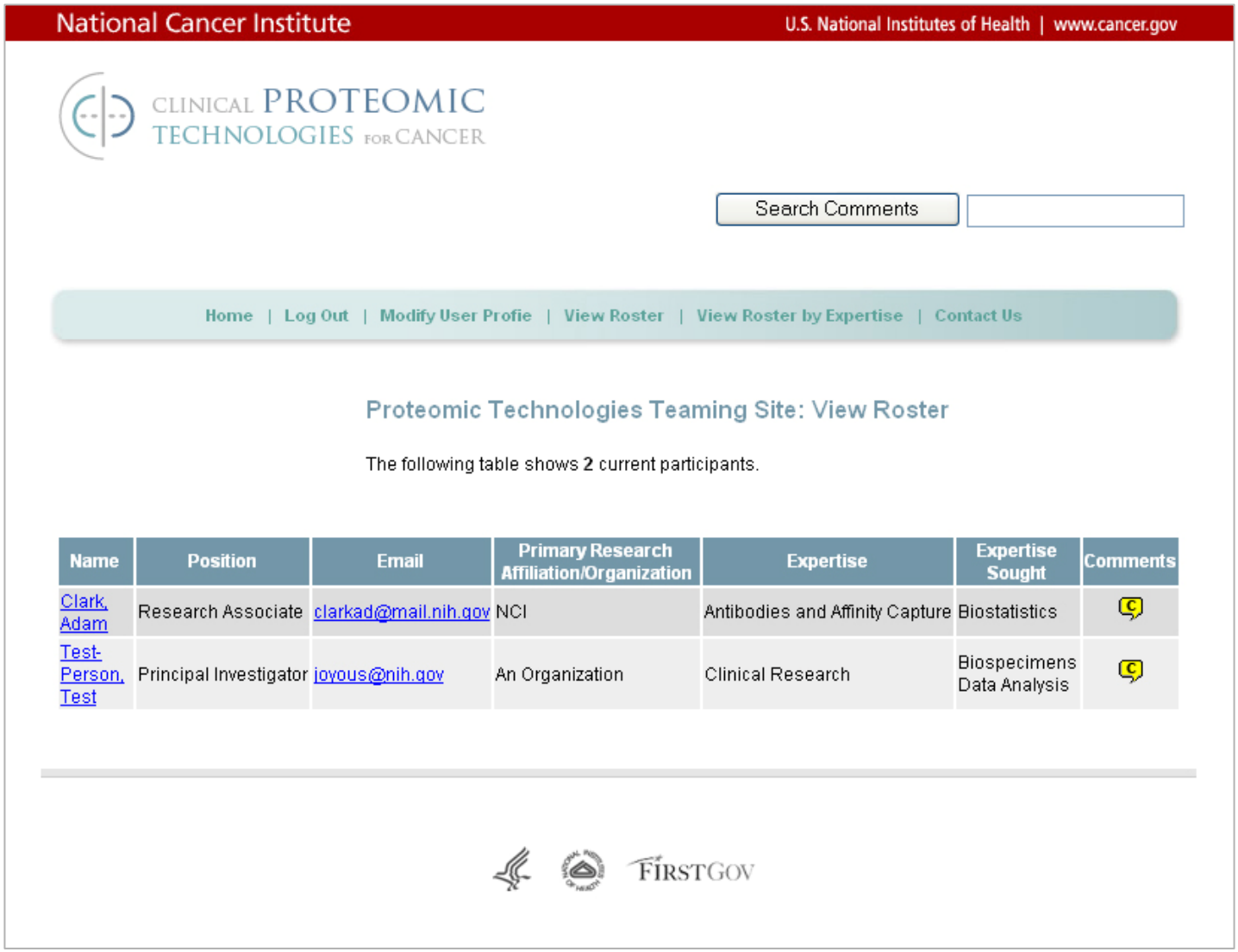
Teaming

- **Experts, technologies, and matching institutes/organizations can identify potential collaborations and team building opportunities through the Teaming Site (http://proteomics.nci.nih.gov/teaming)**
- **Opportunities for PI's, research associates, businesses, and foreign institutes**
- **Collaborations can be established among multiple teams, but the PI may only be included as the PI on one application**

CPTI Teaming Site

- **Web-based system to facilitate initial communications and exploration of collaborative possibilities**
  - Go to: proteomics.cancer.gov
  - Go to “Resource Room”
  - Click on “Proteomic Technologies Teaming Site”
  - Participants submit basic set of information:
    - Position
    - Organization
    - General areas of expertise possessed
    - General areas of expertise sought
    - Comments
- **Participants will then have password access to review other participant information and interests**

CPTI Teaming Site (continued)



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Proteomic Technologies Teaming Site: View Roster

The following table shows **2** current participants.

Name	Position	Email	Primary Research Affiliation/Organization	Expertise	Expertise Sought	Comments
Clark, Adam	Research Associate	clarkad@mail.nih.gov	NCI	Antibodies and Affinity Capture	Biostatistics	
Test-Person, Test	Principal Investigator	joyous@nih.gov	An Organization	Clinical Research	Biospecimens Data Analysis	

FIRSTGOV

Applications

- **Letters of Intent Receipt Date: March 21, 2006**
- **Application Receipt Date: April 21, 2006**
- **Eligible organizations include:**
  - For-profit and non-profit organizations
  - Public or private institutions
  - Domestic institutions and organizations
  - Foreign institutions may participate only as subcontractors
- **Areas of Interest**
- **Application Preparation**

Acknowledgements

- Greg Downing, NCI, OTIR
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- Carolyn Compton, NCI, OBBR
- Jim Vaught, NCI, OBBR
- Mitch Gail, NCI, DCEG
- Ruth Pfeiffer, NCI, DCEG
- Cliff Spiegelman, TAMU
- Sherwood Githens, NCI, DEA
- Chris Hatch, NCI, DEA
- Jan Woynarowski, NCI, DEA
- Tom Stackhouse, NCI, TTB
- Jeffrey Thomas, NCI, TTB
- Ken Buetow, NCI, NCICB
- Peter Covitz, NCI, NCICB
- George Komatsoulis, NCI, NCICB
- Lee Hartwell, FHCRC
- Wendy Law, FHCRC

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Website: http://proteomics.cancer.gov

Email: cancer.proteomics@mail.nih.gov

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